IMMUNOMAGNETIC-ELECTROCHEMILUMINESCENT DETECTION OF *E. COLI* O157:H7 IN GROUND BEEF [1]

C.G. CRAWFORD [2,3], C. WIJEY [3], P. FRATAMICO [4], S.I. TU [3] and J. BREWSTER [3]

[3]*Microbial Biochemistry and Biophysics Research Unit*
USDA/ARS Eastern Regional Research Center
600 East Mermaid Lane, Wyndmoor, PA 19038

[4]*Microbial Food Safety Research Unit*
USDA/ARS Eastern Regional Research Center
600 East Mermaid Lane, Wyndmoor, PA 19038

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ABSTRACT

An immunomagnetic electrochemiluminescence approach using a commercially available instrument was developed to detect the presence of E. coli *O157:H7 in samples of ground beef. The approach can detect* E. coli *O157:H7 that have been incurred with 0.05 CFU of the bacterium per gram of beef after an enrichment for 18 h at 37C. Results of comparison tests indicate that the approach is at least 100 times more sensitive than the Reveal dipstick method with a probable false positive rate less than 4-5%. To enhance the adaptability of the approach, a heat treatment was introduced to eliminate the possibility of generating aerosols of live pathogens during the sample loading process. To minimize the carryover of contaminants associated with the capture of* E. coli *O157:H7 by immuno magnetic beads (IMB), an immuno magnetic separation procedure was developed. The procedure involved the use of a magnetic field to first retain the IMB together with captured bacteria on a paramagnetic column. After washing away the contaminants, the target bacteria were then released from the column by removing the magnetic field. With the proven sensitivity and improved sample treatment procedures, the ECL approach could be considered as an attractive alternative for screening the presence of* E. coli *O157:H7 in foods.*

[1]The mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[2]To whom correspondence should be sent: USDA/ARS, Eastern Regional Research Center, 600 E. Mermaid Ln., Wyndmoor, PA 19038.

INTRODUCTION

Escherichia coli O157:H7 was identified as a food-borne pathogen in 1982 (Riley *et al.* 1983) with outbreaks initially associated primarily with beef and milk (Doyle 1994). Recent estimates from the Centers for Disease Control and Prevention calculate that there are approximately 76 million cases of foodborne illness resulting in 325,000 hospitalizations and 5,000 deaths. Of these cases, *E. coli* O157:H7 is estimated to be responsible for over 60,000 illnesses, 1,800 hospitalizations, and 60 deaths per year (Mead *et al.* 1999). Although undercooked ground beef has been a primary source of the pathogen in foodborne disease outbreaks, *Escherichia coli* O157:H7 has been found in many other foods including pork, lamb, and poultry (Doyle and Schoeni 1987), cider (Besser *et al.* 1993), salami (Rochelle *et al.* 1996), yogurt (Morgan *et al.* 1983), and mayonnaise (Weagant *et al.* 1994).

Rapid detection of *Escherichia coli* O157:H7 in food has become an objective of considerable research effort (Meng *et al.* 1994) with detection methods including ELISA (Padhye and Doyle 1991), PCR (Deng and Fratamico 1996; Gannon *et al.* 1992; Gooding and Choudary 1998), "dipsticks" (Kim and Doyle 1992), epifluorescence (Tortorello and Gendel 1993; Tortorello and Stewart 1994), and Petrifilm™ (Calicchia *et al.* 1994), electrochemical reactions (Abdel-Hammid *et al.* 1999; Brewster and Mazenko 1998), fiber optic biosensor (DeMarco *et al.* 1999), fluorescent bacteriophage (Goodridge *et al.* 1999), quartz crystal microbalance (Spangler and Tyler 1999), and light addressable potentiomeric sensor (Tu *et al.* 1999).

Electrochemiluminescent (ECL) detection of immunomagnetic captured antigens, (Blackburn *et al.* 1991) has been shown to be a rapid and sensitive method to detect a variety of biological materials including, human DNA after PCR amplification, (Schutzbank and Smith 1995; Stern *et al.* 1995; Yu *et al.* 1995) mRNA after PCR amplification, (Vandevyver *et al.* 1995) and inactivated toxins and bacterial spores, (Gatto-Menking *et al.* 1995). This technique has also been reported to be capable of detecting < 2000 cells/mL of heat killed *E. coli* O157 or *Salmonella typhimurium* cells suspended in buffer or in liquids from foods, (Yu 1996; Yu and Bruno 1995). In this study we have expanded on these observations to develop a method to detect *Escherichia coli* O157 in ground beef, and confirm the identification as *Escherichia coli* O157:H7 by immunomagnetic capture and plating on a selective medium.

MATERIALS AND METHODS

Microorganisms

E. coli O157:H7 B1409 and EDL-931 (Centers for Disease Control and

Prevention, Atlanta, GA); *E. coli* O157:H7 ATCC 43890 and 35150 (American Type Culture Collection, Rockville, MD); *E. coli* O157:H7 88-1558 and *E. coli* O157:H⁻ (*E. coli* Reference Center, University Park, PA), and *E. coli* O157:H7 45753-35, 93-437 and ENT C9490 (Food Safety and Inspection Service, Beltsville, MD) were grown for 18 h in brain heart infusion broth (BHI; Difco, Detroit, MI) at 37C with aeration by shaking at 150 RPM. Cells numbers were determined by plating serial dilutions on BHI agar plates and after incubating overnight at 37C, or heat killed *E. coli* O157:H7 (Kirkegaard & Perry Laboratories, Gaithersburg, MD) were counted in a Petroff-Hausser Chamber (Hausser Scientific, Horsham, PA).

Cell Centrifugation

Cells were grown 18 h in BHI then centrifuged at 6,000 × *g* for 10 min. The growth medium was decanted and saved, and the pellet was resuspended in a volume of phosphate buffered saline (PBS) (Sigma, St. Louis, MO) to the original volume of medium. Electrochemiluminescent analysis was then conducted on both samples.

Sample Enrichment

For studies on incurred meat, 25 g samples of ground beef were inoculated with 0.1 mL of PBS containing calculated numbers of *E. coli* O157:H7 and added to 225 mL modified EC broth (Difco Laboratories, Detroit, MI) with novobiocin (Sigma, St. Louis, MO) (mEC+n) in a filter-containing Stomacher bag (Tekmar Co., Cincinnatti, OH). The bag was "stomached" for 2 min, placed in an incubator at 35C, and incubated for 18 h. For analysis of nonincurred meat, 65 g samples of ground beef were placed in 585 mL mEC+n in a filter containing Stomacher bag and treated as described above.

Labeled Antibody Preparation

Ruthenium-labeled antibodies were prepared by adding a 10 M excess of N-hydroxysuccinimide ester of ruthenium (II) tris-bipyridine (IGEN International, Gaithersburg, MD) in dry dimethyl sulfoxide (Sigma, St. Louis, MO) to 0.3 - 0.5 mg goat antibody to *E. coli* O157:H7 (Kirkegaard & Perry Laboratories, Gaithersburg, MD) in 300 µL PBS. The mixture was incubated at room temperature for 60 min in the dark, then the reaction was terminated by adding 20 µL of 2.0 M glycine (Sigma, St. Louis, MO) and incubating for an additional 10 min. The reaction mixture was transferred to a Sephadex G-25M PD-10 column (Pharmacia Biotech, Uppsala, Sweden) and eluted in 0.5 mL fractions with PBS, containing .05% sodium azide.

IM-ECL Analysis

Routinely, 0.1 mL of the enriched sample was added to 0.9 mL PBS in 12 x 75 mm polypropylene round bottom tubes (Sarstedt, Newton, NC) and heated for 10 min at 95C. We constructed an insert for a commercial heating apparatus by cutting an aluminum block to fit the well of the heating mantle, and drilling the block with 50, 13 × 15 mm round bottom holes to match the bottom of the tubes. When the samples were placed in the heating block, capping the tubes was unnecessary because the 60 mm of the tube above the heated block, acted as a condenser. The sample was filtered through a 300 μ polypropylene filter (Fisher Scientific, 11-387-50, Pittsburgh, PA) into a new 12 × 75 mm polypropylene round bottom tube. After the sample had cooled to room temperature, 100 ng of ruthenium-labeled antibody, and 2.5 μL of anti-*E. coli* Dynabeads (Dynal Corp., Lake Success, NY) in 50 μL of a PBS blocking buffer, pH 7.4, containing 2% blotting grade goat serum (Rockland, Gilbertsville, PA) and 2% Tween 20 (Sigma, P2287, St. Louis, PA) was added. The tubes were vortexed on the ORIGEN analyzer (IGEN International, Gaithersburg, MD) carousel at 100 RPM for 60 min and the ECL measured by the ORIGEN instrument.

EHEC-TEK ELISA Analysis

Stationary-phase *E. coli* O157:H7 cells were centrifuged at 6,000 × *g* for 10 min to separate the growth medium and cells. The cells were resuspended in a volume of PBS equal to the original volume and 10-fold serial dilutions were made. The saved growth medium was also diluted in a similar manner and 100 μL portions from both cell and supernatant dilutions were used for analysis by a EHEC-Tek system (Organon Teknika Inc., Durham, NC) according to the manufacturer's instructions.

Reveal Analysis

After warming to room temperature, REVEAL *E. coli* test devices (Neogen, Lansing MI) were used to analyze samples. Following the manufactures instructions, 0.12 mL of sample was applied to the sample port and the test results were recorded after 15-20 min.

Immunomagnetic Cell Capture

One mL of the enriched sample was put in a sterile microfuge tube and 5 μL of anti-*E. coli* Dynabeads in 45 μL of blocking buffer was added. The tube was gently rotated on a Labquake Shaker (Labindustries, Inc., Berkely, CA) for 10 min.

Immunomagnetic Cell Separation (IMS)

MACS Large Cell Separation Columns (Miltenyi Biotec, Auburn, CA) were placed in an OctoMACS Separation Magnet (Miltenyi Biotec, Auburn, CA) and the column was washed with 1 mL of a column buffer (CB), buffered peptone water (BPW) containing 2 mM EDTA (Sigma, St. Louis, MO) and 0.5% BSA (Sigma, St. Louis, MO). The immunomagnetic cell captured sample was applied to the column and allowed to run into the paramagnetic bed then the column was washed three times with 3 mL of CB, allowing the CB to flow into the column before applying the next volume of CB. The column was removed from the magnet and the cells were gently flushed from the column by 1 mL of CB, using the sterile plunger supplied, into a sterile microfuge tube.

Plating Samples on Selective Media

Rainbow Agar O157 media (Biolog, Hayward, CA) or MacConkey sorbitol agar (Difco, Detroit, MI) containing 0.1 g 5-bromo-4chloro-3-indoxyl-β-D-glucuronide per l (Biosynth International, Skokie, IL) (MSA-BCIG)(Johnson *et al.* 1995; Okrend *et al.* 1990) was prepared and poured into 10 cm plates. Aliquots of 0.1 mL from the samples eluted from the MACS column were spread on the plate, and in some cases, the remaining 0.9 mL was centrifuged at 6,000 × g for 1 min to remove the buffer, and the bead-captured material was resuspended in 0.1 mL of BPW for plating.

RESULTS AND DISCUSSION

Electrochemiluminescence Analysis Using The ORIGEN Instrument

The IGEN International ORIGEN system used in this study is comparatively new (Blackburn *et al.* 1991; Deaver 1995; Jameison *et al.* 1996) and contains 3 main components, sample loading carousel, luminescence detector and a computer controller. The luminescence origin is the electrochemical reactions associated with a protein-bound ruthenium (Rh) complex. In experiments, a N-hydroxysuccinimide ester of Rh(II)tris-bipyridine chelate is first used to react with an antibody to produce Rh(II)-antibody conjugates. The conjugates are then used to capture the antigen-containing material in solutions. Immunomagnetic beads (IMB) coated with the same or a different antibody targeted to the antigen (IMB) are then applied to further label the antigen to form Rh-antigen-IMB complexes. The complexes, in the reaction tubes on a carousel, are drawn into a flow of tripropylamine (TPA) containing buffer that passes over an electrode. A magnet positioned directly under the electrode retains the complexes and unreacted IMB on the electrode and allows fresh buffer to wash away other components in sample

solutions. Electric voltage is then applied to the electrode to oxidize both TPA and Rh(II). Being a strong reductant, the oxidized TPA radical reduces the Rh(III) back to Rh(II) but at an excited state. As the excited species relaxes back to its ground state, it emits light at 620 nm. A photon counting system mounted directly above the electrode is used to detect and integrate emitted light. The integrated light intensity provides a quantitative measure of the amount of the antigen captured by the IMB. The sample carousel holds 50 tubes, and the instrument takes about 1 min to read each tube. Thus, with the reaction incubation time as 60 min in sample tubes, fifty samples can be analyzed in ~ 2 h.

Attempt to Enumerate *E. coli* O157:H7 from Observed ECL Signal

The above ORIGEN instrument has been applied to detect *E. coli* O157:H7 in simple solutions (Yu and Bruno 1995; Yu 1996). We have extended its applications to detect *E. coli* O157:H7 spiked in ground beef. The data (not shown) indicated that substantial ECL signals could be observed from samples spiked with 1 CFU/g after a brief enrichment (5 to 6 h at 37C). However, attempt to quantitatively enumerate the bacteria from observed ECL signal yield poor and variable correlations. To determine the possible origin of these results, we centrifuged the bacterial samples to collect both the cell pellets and the supernatant from seven strains of *E. coli* O157:H7 and an H-minus strain. ORIGEN analysis of both fractions yielded results shown in Table 1. In all the *E. coli* O157:H7 strains tested, more ECL activity was found in the supernatant fractions. ECL activity was also found in the cell pellet and supernatant of the H-minus stain. This is expected since the polyclonal antibodies used to coat the IMB are specific to O157 only.

To test whether the observations described in Table 1 could be found by other immuno methods, we analyzed the cell and supernatant fractions of *E. coli* O157:H7 (B1409) with the Organon Technika EHEC-Tek kits. Positive results, as depicted in Table 2, were obtained. In another experiment, ground beef incurred with *E. coli* O157:H7 was enriched for 18 h. The obtained cell and supernatant were serially diluted and analyzed by the Reveal dipsticks. Again, both fractions showed positive results (Table 3). Thus, tests by all three different methods, using samples obtained from either enriched ground beef or pure culture cells at stationary phase, indicated the presence of antigenic materials in the supernatant. It should be noted that the release of antigenic materials to the same polyclonal antibodies in supernatant of pure or mixed culture of growing *E. coli* O157:H7 was detected by the Organon Technika EHEC-Tek kit (Padhye and Doyle 1991). While the exact origin of the antigens found in the supernatant remain to be established, it is of interests to note that *E. coli* can release small vesicles composed of cell outer membranes (Hoekstra *et al.* 1976; Gankema *et al.* 1980).

TABLE 1.
PERCENTAGE OF TOTAL ECL ACTIVITY ASSOCIATED WITH CELLS AND GROWTH MEDIUM FROM VARIOUS *E. COLI* STRAINS

Strain	Cells	Supernatant
O157:H7 (45753-35)	25.2	74.8
O157:H7 (93-437)	26.2	73.8
O157:H7 (EDL-931)	25.4	74.6
O157:H7 (30-2C4)	17.0	83.0
O157:H7 (88-1588)	27.6	72.4
O157:H7 (43890)	40.7	59.3
O157:H7 ENT C9490	14.4	85..6
O157:H⁻	29.0	71.0

TABLE 2.
EHEC-TEK ANALYSIS OF CELLS AND GROWTH MEDIUM SUPERNATANT FROM CULTURES OF *E. COLI* O157:H7 B1409. VALUES ARE ABSORBANCE AT 450 NM

Cells/ml	Cells	Supernatant
8.9×10^7	> 3.0	> 3.0
8.9×10^6	> 3.0	1.837
8.9×10^5	1.243	0.263
8.9×10^4	0.164	0.094
8.9×10^3	0.085	0.066
8.9×10^2	0.065	0.057
	Negative Control	0.054
	Negative Control	0.050
	Positive Control	> 3.0

TABLE 3.
REVEAL ANALYSIS OF CELLS AND SUPERNATANT FROM ENRICHED *E. COLI* O157:H7 INCURRED SAMPLES OF GROUND BEEF

Dilution	0	10^{-1}	10^{-2}	10^{-3}
A – Cells	+	+	+/-	-
A – Supernatant	+	+	+	-
B – Cells	+	+	+	-
B – Supernatant	+	+	+	+/-

\+ Strong Positive Reaction
+/- Weak Positive Reaction
\- Negative reaction

From a practical point-of-view, the described results indicate that the ECL signal may not be used to accurately enumerate cell counts of the *E. coli*. The small vesicles captured by the IMB would be just active as the whole bacteria in yielding the ECL signals. This effect can not be eliminated without a knowledge on the relative concentrations of the vesicles and the intact cells in a given sample. However, the added ECL signal of cell fragments may enhance the detection sensitivity to the presence of targeted bacteria.

Heat Treatment of *E. coli* O157:H7 Prior to ECL Measurement

The above discussion suggests that the ECL signal may be increased by fragmenting the bacteria prior to IMB capture. Since the sample tubes were constantly agitated by vortex motion in the loading carousel, concerns about generating aerosols of live *E. coli* O157:H7 should also be addressed. Thus, we introduced a heating step prior to load the samples to the carousel. Fifty bacterial samples of 1 mL each in volume were placed in 12 × 75 mm polypropylene tubes inserted in 13 × 15 mm round bottom holes in an aluminum heating block. The samples were heated to 100C for 10 min and then transferred to the sample carousel for testing. As shown in Fig. 1, the heating process induced a three-fold increase in ECL signals. Apparently, heating the cells fragmented them and increased the capture effectiveness of the IMB. The data of Fig. 1 also indicated that the ECL method of ORIGEN can be used to detect samples containing less than 10^3 cells/mL. The heating process also produced flocky precipitate that occasionally blocked the fine diameter tubing in the instrument. Thus, a filtration step was introduced using 300 μ disposable polypropylene filters. As shown in Fig. 2, this filtration step did not significantly decrease the ECL signals suggesting the majority of the IMB-positive antigens were small in size after the heat treatment.

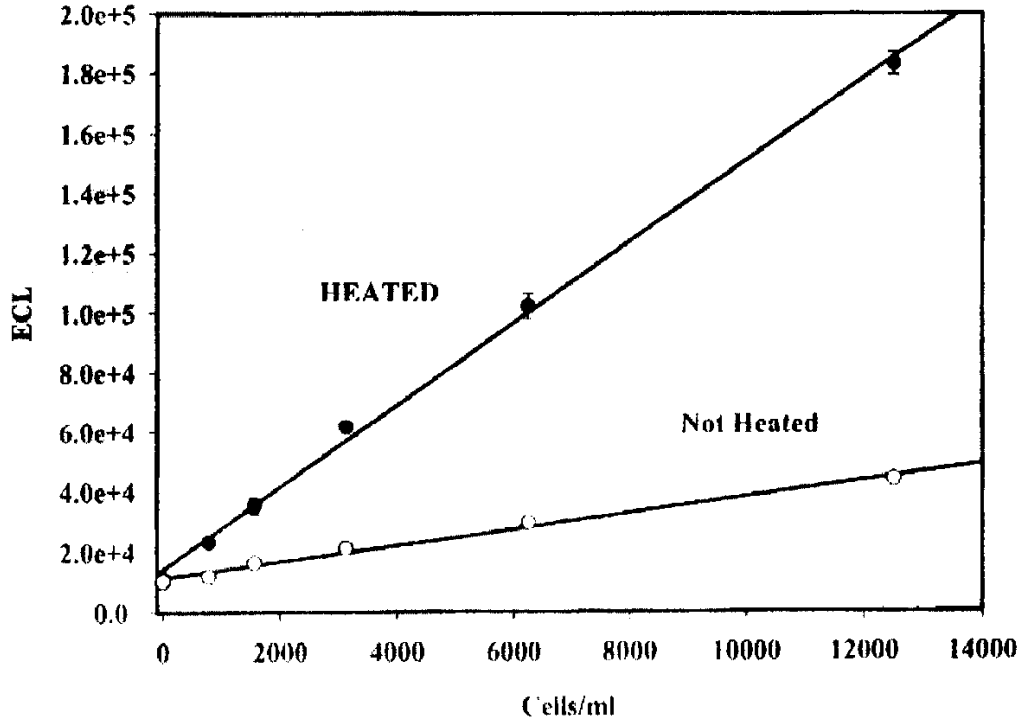

FIG. 1 EFFECT OF HEAT-TREATING A SAMPLE AT 95C FOR 10 MIN

E. coli O157:H7 (B1409) were grown 18 h in BHI then diluted in PBS to 200,000 cells/mL then diluted 1:1 in PBS to the levels indicated in the graph. ECL analysis was conducted on triplicate samples at each. One set of samples was analyzed directly and the other heated at 95C for 10 min before analysis.

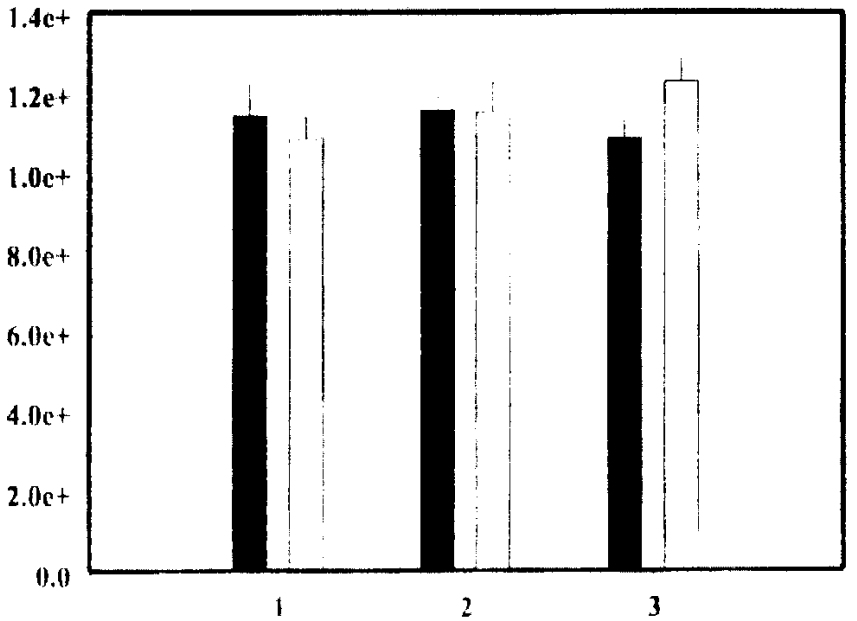

FIG. 2. ECL ACTIVITY OF 2 X 10^5 *E. COLI* O157:H7 (B1409) CELLS FILTERED AND UNFILTERED AFTER HEAT-KILLING

Dark bars are unfiltered samples, light bars are filtered samples.

Sensitivity Comparison Between ORIGEN and Other Detection Processes

One of the common applied *E. coli* O157:H7 test method is the Reveal dipstick analysis. We compared the relative sensitivity between the Reveal test and the ECL approach. Samples of enrichment broth from nonincurred ground beef were spiked with four different strains of *E. coli* O157:H7 then 10 fold serial dilutions were made of each sample. ECL and Reveal were conducted on each sample and immuno magnetic separation was conducted on the Reveal negative samples for further tests.

Immunomagnetic capture of *E. coli* O157:H7 has been used to isolate targeted pathogen from enriched meat samples (Fratamico *et al.* 1992; Okrend *et al.* 1992), using a time-consuming procedure that involved three centrifuge-resuspension washing steps to reduce the carryover of nontarget contaminants. To reduce the washing time, we applied IMS procedure to positively select IMB captured pathogens using a paramagnetic column in a manner similar to that for eukaryotic cell isolation (DiNicola *et al.* 1996; Garau *et al.* 1997; Schmitz *et al.* 1994). Aliquots of 1 mL in volume of Reveal-negative samples were treated with IMB and then subjected to IMS as described in Material and Methods. Portions (0.1 mL) of the finally collected IMB-containing eluents were plated on Rainbow agar. The remaining 0.9 mL portions were centrifuged at 6,000 × g for 1 min and obtained pellets were resuspended in 0.1 mL aliquots of BPW before being plated.

The data in Table 4 revealed that more than 100 fold dilution of enriched samples, effectively abolished the sensitivity of the dipstick analysis of Reveal. However, substantial ECL signals and sufficient colony counts by Rainbow agar were obtained in samples after 10,000 folds dilution. Thus, the ECL approach is at least 100 times more sensitive than the Reveal method in detecting live *E. coli* O157:H7. However, because of the presence of small antigen-containing vesicles, no good correlation between CFU and ECL signal intensity was observed. It should be mentioned that the heat treatment step was omitted in all ECL experiments described in Table 4.

Reliability of the ECL Approach

The results of Table 4 suggested that an ECL signal above 25,000 could be a good indicator for the presence of *E. coli* O157:H7. During the course of our study, we analyzed more than 1,100 nonincurred beef samples using the ECL method. Only a few samples required standard culture/biochemical confirmation test for the presence of *E. coli* O157:H7. Similarly, less than 4 and 5% of the samples yielded ECL signals greater than 50,00 and 25,000, respectively (Table 5). Thus, if we arbitrarily choose ECL signal of greater than 50,000 as presumptive positive for *E. coli* O157:H7, the maximum false positive rate is still less than 4%.

To further demonstrate the advantage of the ECL method, we incurred ground beef with extremely low levels of *E. coli* O157:H7. Forty eight ground beef

TABLE 4.
REVEAL, ORIGEN, AND RAINBOW AGAR PLATING ANALYSIS OF INCURRED GROUND BEEF

Sample	Method	10^{-2}	10^{-3}	10^{-4}	10^{-5}
A - 1	Dipstick	+	-	-	-
	ECL	648,962	623,995	526,900	4,313
	Plating		28, 200	3, 20	0, 2
A - 2	Dipstick	+	-	-	
	ECL	700,806	246,189	39,068	4,664
	Plating		14, 100	1, 10	0, 2
A - 3	Dipstick	+	-	-	
	ECL	670,438	177,874	26,394	3,305
	Plating		11, 200	1, 18	0, 3
A - 4	Dipstick	+	-	-	
	ECL	360,239	77,817	9,740	1,949
	Plating		3, 30	1, 3	0, 0
B - 1	Dipstick	+	+/-	-	
	ECL	525,734	472,364	116,868	14,463
	Plating			5, 42	0, 4
B - 2	Dipstick	+	+/-	-	
	ECL	691,662	366,858	65,685	8,780
	Plating			3, 18	1, 0
B - 3	Dipstick	+	-	-	
	ECL	575,006	405,073	83,444	9,873
	Plating		55, 300	5, 38	1, 5
B - 4	Dipstick	+	-	-	
	ECL	647,694	262,918	42,422	5,191
	Plating		20, 50	1, 13	0, 2

A and B are enrichment broths; 1-4 are strains of *E. coli* O157:H7; the two plating numbers, represent the number of *E. coli* O157:H7 colonies found on Rainbow agar when 0.1 ml and 0.5 ml of the sample were applied to the plate.

TABLE 5.
ECL ANLYSIS OF 65 GRAM GROUND BEEF SAMPLES

Run	ECL < 50,000	ECL > 50,000	ECL>25,000
1	55	2	4
2	100	2	2
3	86	5	6
4	88	5	7
5	80	3	3
6	95	0	0
7	82	1	3
8	65	0	2
9	111	1	1
10	105	9	9
11	97	0	0
12	87	8	9
13	82	6	7
Total	1,133	42	53

samples, 25 g each, were spiked with 0.05 or 0.5 CFU/g of the bacteria and then enriched in 225 mL mEC+n medium for 18 h. Reveal test and ECL approach (without heat treatment) were then applied to each samples. After immunomagnetic cell capture and separation, all IMB samples were plated on MSA-BCIG and some of the samples were also plated on Rainbow agar. Of the 38 samples that were positive for *E. coli* O157:H7 on MSA-BCIG plates, 87% of those samples had an ECL signal above 50,000 and only less than 45% showed positive to Reveal test (Table 6).

CONCLUSION

In this work, we summarized the advantages and the pitfalls of the ECL method using ORIGEN system. The results demonstrated conclusively that the ECL approach is more sensitive and reliable than the Reveal test for the presence of live, injured and fragmented *E. coli* O157:H7 in beef samples. However, the method can not be used to reliably enumerate the CFU of the targeted bacteria. We have also developed a heat treatment and an IMS procedure to alleviate the aerosol concerns and to speed up the sample preparation, respectively. These advancements and improvements should make the described ECL approach, an attractive alternative for screening the presence of pathogenic *E. coli* O157:H7 in foods.

TABLE 6.
ECL, REVEAL, AND MSA-BCIG, AND RAINBOW PLATING ANALYSIS OF 25 G SAMPLES OF GROUND BEEF INCURRED WITH 0.05 (VL) AND 0.5 CFU (L) PER GRAM

Sample	ECL	REVEAL	MSA-BCIG	Rainbow
8 VL, 2 L	1550-4330	NEG.	-	*
3 VL	5-25,000	NEG.	+	**
2 VL	25-50,000	NEG.	+	ND
- Control	10,229	NEG.	-	ND
VL	70,037	NEG.	+	ND
VL	89,472	NEG.	+	+
VL	127,941	NEG.	+	+
VL	129,079	NEG.	+	ND
L	144,668	NEG.	+	+
L	177,142	NEG.	+	ND
L	179,356	NEG.	+	ND
+ Control	202,957	POS.	+	ND
VL	217,972	NEG.	+	ND
VL	226,888	NEG.	+	+
L	274,462	NEG.	+	+
L	294,623	NEG.	+	+
L	300,166	NEG.	+	ND
VL	316,817	NEG.	+	+
VL	327,830	NEG.	+	+
L	334,300	NEG.	+	+
L	384,641	NEG.	+	+
4 VL, 13 L	<400000	POS.	+	***

* Four samples were tested and all were negative
** Two samples were tested and both were positive
*** Ten samples were tested and all were positive
ND Not determined
\+ *E. coli* O157:H7 positive

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